Mitochondria possess their own DNA (mtDNA) which is organized in specific complexes called nucleoids. Each nucleoid contains several copies (~6 in human) of mtDNA associated with “nucleoid” assessor proteins, such as mitochondrial transcription factor A (TFAM), which is literally coating mtDNA; and execution proteins participating in transcription (e.g. mitochondrial single-stranded DNA-binding protein, mtSSB) and translation (e.g. polymerase gamma). Functional consequences of this intricate organization are not yet fully understood.

Rotenone induces maximum superoxide production on mitochondrial Complex I by blocking electron transport which inhibits cell respiration. The latter also leads to fission of mitochondrial reticulum network to short segments and small spheres [1]. Thus not only oxidation and subsequent mutation of mtDNA molecules can occur upon rotenone treatment, but also redistribution of mt nucleoids in fragments of mitochondrial network. These phenomena were investigated by conventional confocal microscopy and examples were confirmed by BiplaneFPALM microscopy. Nucleoids were visualized either by a coating protein used as a marker (expression of TFAM-EGFP or TFAM-Eos for BiplaneFPALM) or using SYTO16 staining of mtDNA. We demonstrate that rotenone treatment leads to decrease in number and increase in size of mt nucleoids in HEPG2 cells. Rotenone leads to increase in size of mt nucleoids as observed with TFAM-EGFP. The most remarkable difference was observed with 80nM Rotenone when mitochondrial network was fully fragmented. When 20 nM (48 h) or 40 nM (24 h) Rotenone incubation was used, mt nucleoids appeared bigger in size although mitochondrial tubules remained mostly connected. Rotenone treatment of HEPG2 cells led also to decrease in number of mt nucleoids. The effect was most pronounced after 48 h incubation with 20 nM Rotenone.